DETOXIFICATION OF CHEMICAL WARFARE AGENTS BY THE PLANT CHOLINERGIC SYSTEM

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ABSTRACT

Plants have cholinesterases (pChEs), anti-ChEs, and activators of ChEs. We have isolated pChE from mung bean sprout. We investigated 300 plants and found that 75% of them contained anti-ChE that inhibited ChEs. Thirty-five percent contained activators of ChEs. An activator of ChE from wheat leaf, "Tritiacche-T123", activates fetal bovine serum AChE, equine BChE, and pChE. This non-oxime natural plant product may offer a new approach to the reactivation of OP-inhibited ChEs. pChE showed substrate differences from human acetylcholinesterase (AChE) or butyrylcholinesterase, hydrolyzing propionylthiocholine > acetylthiocholine > butyrylthiocholine. pChEs might also be useful as bioscavengers of OP nerve agents.

INTRODUCTION

Organophosphate chemical warfare agents (CWA) and pesticides are extremely poisonous compounds. Nerve agents irreversibly inhibit acetylcholinesterase (AChE) activity at synapses, stop neuronal signal transmission, and destroy the cholinergic system machinery and can ultimately cause death. Reactivation of inhibited AChE by traditional oxime reactivators (for example 2-PAM, LÜH6, TMB4, and HI-6,) is an efficient way to attenuate toxicity and thus plays a key role in the treatment of nerve agent poisoning. However, their efficacies can be limited due to either poor or non-reactivation of the CWA-inhibited cholinesterases (ChEs), most notably in the case where ChEs have been exposed to soman (GD) because it "ages" rapidly. New activator/reactivators[1] could be useful to overcome these difficulties.

Plants also contain anti-ChEs, for example physostigmine and huperzine A[1,2]. Pyridostigmine is a synthetic analogue of physostigmine[3], a natural product from the plant *Physostigma venunosum,* and is used therapeutically for a variety of diseases including myesthenia gravis.

Among the enzymes being considered and evaluated as bioscavengers of organophosphate (OP) nerve agents are the cholinesterases (ChEs), specifically human serum butyrylcholinesterase[4], the OP hydrolases (bacterial sources), and carboxylesterase. An effort has been made to find new ChE candidates as bioscavenger. Interestingly, plants have been found to have cholinesterase(s)[5]. Therefore we have isolated and partially purified a plant ChE from mung bean sprouts. These biological compounds from plants may be useful as bioscavengers and protectants of OPs, and activators of OP-poisoned enzymes.

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METHODS

Isolation and Characterization of plant activator(s): Wheat (*Triticum aestivum*) leaves were grown in laboratory. The methanol extract of wheat leaves was analyzed for its various chemical constituents by thin layer chromatography (TLC), normal phase column chromatography (silica), and HPLC. TLC plates (20 x 20 cm), coated with silica gel G (0.25 mm thickness), were pre-dried and then loaded with the methanolic solution. The plates were developed in a variety of solvent systems. Fractions were analyzed for their ability to increase (activate) AChE activity in the Ellman assay[6]. The band that activated AChE the most had an Rf of 0.53 in chloroform: methanol (1:4) system. This material exhibited bright blue fluorescence under UV illumination. The activity was concentrated under vacuum, and then re-chromatographed on a silica gel column in water: methanol (1:3). The active fraction (Rf 0.94) was once again re-re chromatographed on the silica column in water: methanol (1:3). These fractions are being evaluated for purity and structural elucidation by HPLC, NMR, and MS.

Purification of plant cholinesterase (pChE): pChE was extracted from the roots of mung bean sprouts (*Phaseolus aureus*). The roots were crushed and homogenized in 0.1 M sodium phosphate buffer pH 8; 2:1, (v/w), and extracted by stirring for 1 h in 1% Triton X and 1 M NaCl. The remaining insoluble material was removed by filtration through a nylon mesh. The filtrate was centrifuged at 15,000 x g for 30 min at 4 C. The resulting supernatant was dialyzed against 5 mM sodium phosphate buffer pH 8. The protein fraction was concentrated by using polyethylene glycol. Next, the enzyme was applied to a procainamide affinity gel column for purification. Active fractions (1 ml each) were collected, and pooled prior to use.

RESULTS AND DISCUSSION

ChEs inhibited by certain OPs can become refractory to spontaneous or oxime induced reactivation because the enzyme-OP has under gone dealkylation or "aging". Alternatively, some enzyme: OP respond very slowly to oxime reactivation. For these reasons, there has been a renewed interest in novel oxime or non-oxime reactivators for nerve agent poisoning.

Anti-cholinesterase compounds (anti-ChEs) are widespread in plants as shown in Table 1. On the other hand, plants were also found to be a rich source of compounds that can act as ChE activators as shown in Table 2. Purified plant derived activators may be of value as a treatment against organophosphate poisoned ChEs.

Plant Activators of Cholinesterase: We found that an extracted from wheat leaves, such as "Tritiacche-T123" and other compounds from plants activated fetal bovine serum AChE up to three fold (Fig.1), and also activated equine BChE and pChE cholinesterases. Two novel partially purified compounds that activate eel AChE were isolated from wheat leaves, Triticheac[1] and Whecheac[1]. A third activator of ChE from wheat leaf, "Tritiacche-T123" is three-fold more potent than Triticheac. These non-oxime natural plant products may offer a new approach to the reactivation of OP-inhibited ChEs. The mechanism by which Tritiacche-T123 activated purified fetal bovine serum AChE three-fold is currently being investigated.

Table 1. Survey of plants for anti-ChE Activity				
Division	**Families**	**Families tested positive**	**Species**	**Species tested positive**
Algae	**8**	**6**	**19**	**10**
Fungi	**4**	**4**	**13**	**12**
Lichens	**4**	**4**	**6**	**6**
Bryophytes	**11**	**3**	**18**	**4**
Pteridophytes	**12**	**12**	**38**	**35**
Gymnosperms	**10**	**10**	**18**	**16**
Angiosperms	**112**	**86**	**191**	**145**
Total	**161**	**125**	**303**	**228**

Table 2. Survey of plants for activator(s) of AChE [Acti-ChE]				
Division	**Families**	**Families tested positive**	**Species**	**Species tested positive**
Algae	**8**	**7**	**19**	**9**
Fungi	**4**	**1**	**13**	**3**
Lichens	**4**	**0**	**6**	**0**
Bryophytes	**11**	**10**	**18**	**14**
Pteridophytes	**12**	**6**	**38**	**9**
Gymnosperms	**10**	**3**	**18**	**2**
Angiosperms	**112**	**38**	**191**	**69**
Total	**161**	**65**	**303**	**106**

Plant cholinesterase: Interestingly, plants have been found to contain ChEs, and these enzymes have been proposed as a bioscavenger for OPs[4]. We have examined ChE activity derived from mung bean roots. This partially purified pChE showed substrate specificity different than mammalian acetylcholinesterase, hydrolyzing thiocholine substrates in the following order: propionylthiocholine > acetylthiocholine > butyrylthiocholine (Fig. 2). Based on acryl amide gel electrophoresis analysis, the pChE appears to be a dimer (~120 kD).

These natural pChEs might be useful for immobilizing on polyurethane sponge used for decontamination of skin and wounds and as a pretreatment for OP poisoning, if it is compatible with human system.

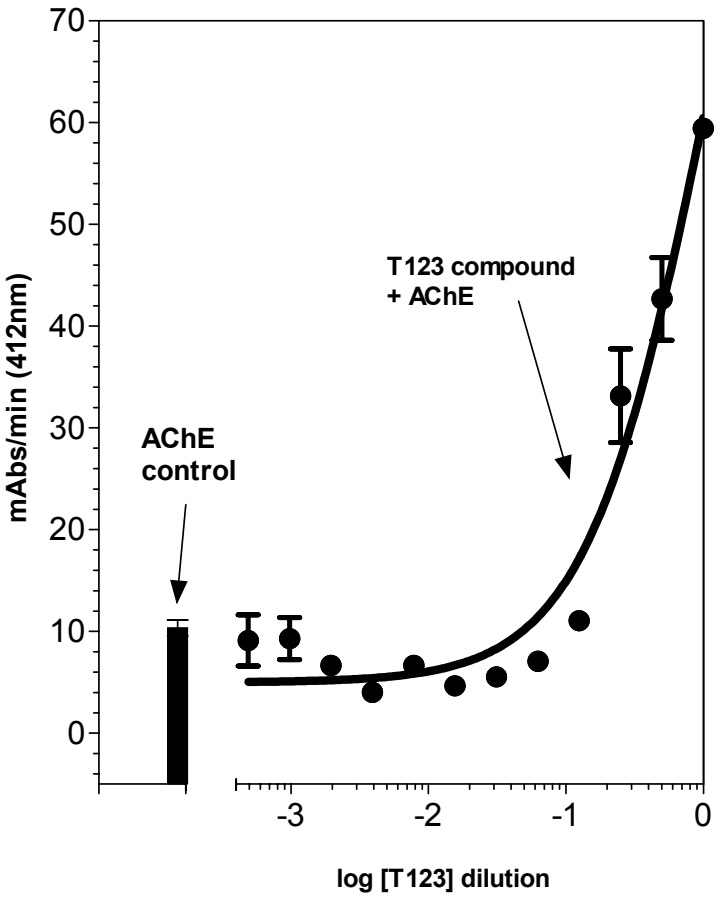
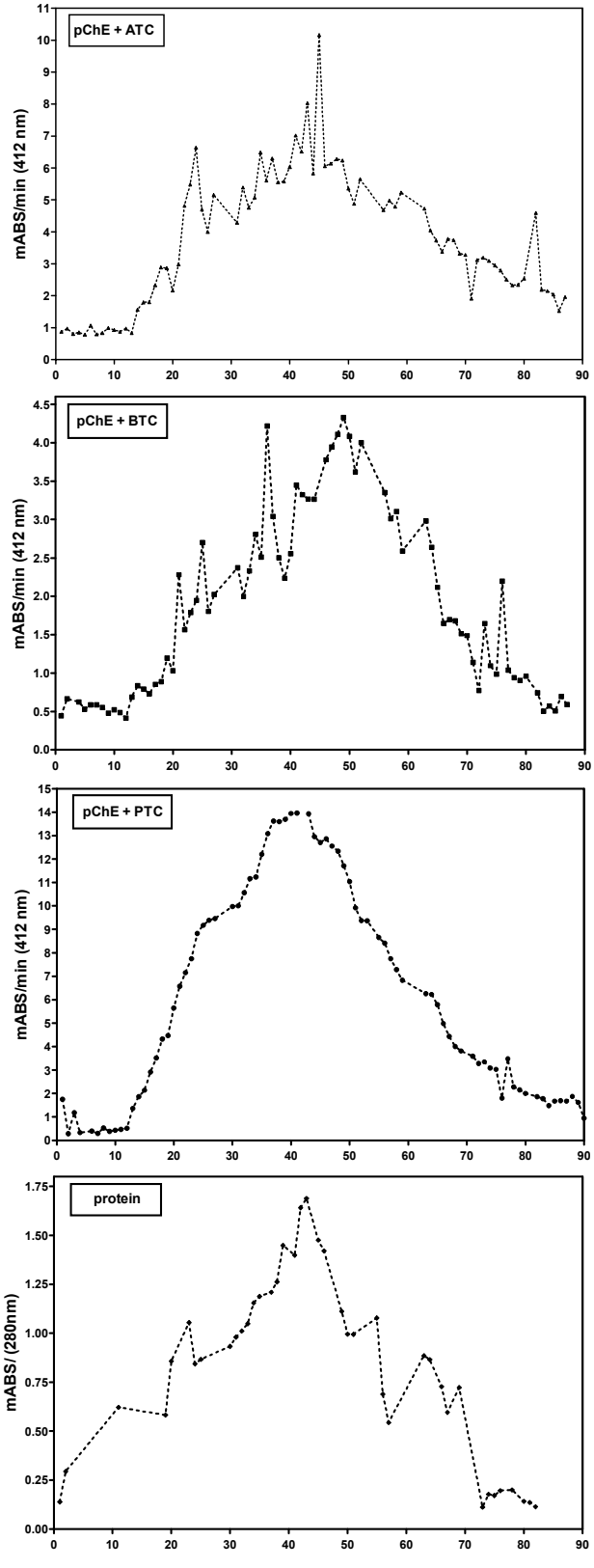


Fig. 1. Effect of Tritiacche-T123 compound on FBS AChE

Fig. 2. Plant ChE (pChE, from mung bean)
Hydrolysis of ATC, BTC, & PTC and protein concentration after procainamide column affinity purification



CONCLUSION

- Plants have cholinesterase and anti-cholinesterase and novel activator(s) of ChEs.
- A partially purified compound from wheat, "Tritiacche-T123", activated fetal bovine serum AChE up to three-fold.
- These non-oxime natural plant AChE activating products may offer a new approach to the activation/reactivation of OP-inhibited ChEs.
- Based on acryl amide gel electrophoresis analysis, the pChE appears to be a dimer (~120 kD).
- This partially purified pChE showed substrate specificity different than mammalian acetylcholinesterase, hydrolyzing thiocholine substrates in the following order: propionylthiocholine > acetylthiocholine > butyrylthiocholine.
- pChE may be useful for the decontamination of OPs and as a bioscavengers for OPs, thereby preventing or decreasing OP poisoning.

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